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<b>(21) International Application Number:</b> PCT/GB96/01082 <b>(22) International Filing Date:</b> 7 May 1996 (07.05.96) <b>(30) Priority Data:</b> 9509263.1                      5 May 1995 (05.05.95)                      GB 9607505.6                      11 April 1996 (11.04.96)                      GB <b>(71) Applicants (for all designated States except US):</b> IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE [GB/GB]; Sherfield Building, Imperial College, London SW7 2AZ (GB). LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE [GB/GB]; Keppel Street, London WC1E 7HT (GB). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> MILLER, Andrew, David [GB/GB]; 27 Fairlawn Grove, Chiswick, London W4 5EJ (GB). RAYNES, John, Graham [GB/GB]; 112 St Albans Road, Sandridge, St Albans AL4 9LJ (GB). <b>(74) Agents:</b> CHAPMAN, Paul, William et al.; Kilburn & Strode, 30 John Street, London WC1N 2DD (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> ANTISENSE PEPTIDES  <b>(57) Abstract</b>  <p>Antisense peptides are provided which are useful in antagonising the biological effects of target molecules. In particular, the target molecule is a cytokine, e.g. IL-1<math>\alpha</math> or IL-1<math>\beta</math>, TNF<math>\alpha</math> or IL-8 and the antisense peptides thus find use in treating or preventing conditions mediated by these cytokines, for instance inflammatory conditions or cancer.</p>		

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## ANTISENSE PEPTIDES

5 The present invention relates to antisense peptides or polypeptides capable of binding to a target ligand. In particular, it relates to antisense peptides which will bind to biologically active molecules such as IL-1 $\alpha$  and/or IL-1 $\beta$ , TNF and Eotaxin, thereby altering their biological activity, and their use in medicine.

10 The reason why DNA is an anti-parallel double helix has traditionally been thought to derive from the requirement for replication of the genetic code. The sense strand provides the code and the antisense the means of propagating that code. However, another relationship is  
15 now thought to exist between sense and complementary /antisense strands which suggests a more substantive role for antisense DNA. Blacock and Smith (*Bioch.Biophys. Res.Comm.*, 121:203 (1984)) observed that antisense DNA was able to code for peptides which are essentially the  
20 hydropathic complement of those dictated by sense DNA. This observation was followed by the subsequent discovery that sense peptides and their hydropathically complementary antisense peptides are able to interact (Bost et al, *PNAS USA*, 82:1372 (1985)).

25 This later observation has now been elaborated into a Molecular Recognition Theory (MRT) to account for the interactions of protein ligands with their receptor targets and which has been used to explain a variety of  
30 interactions including IL-2 with its receptor (Weigent et al, *Bioch.Biophys.Res.Comm.*, 139:367 (1986); Bost et al, *Bioch.Biophys.Res.Comm.*, 128:1373 (1985)), collagen with collagenase (de Souza and Brentani *J.Biol.Chem.*, 267:13763 (1992)) and cystatin C with C4 (Ghiso et al,

PNAS USA, 87:1288 (1990)).

5 Furthermore, a recently described method for the affinity  
purification of IL-1 $\beta$  from complex synthetic mixtures and  
cell lysates (Fassina et al, *Int.J.Peptide Protein Res.*,  
39:549 (1992); Fassina & Cassani, *Biochem.J.*, 282:773  
(1992)) employed a polypeptide designed to act as a  
complement to a specified region of IL-1 sequence to bind  
10 the protein with high affinity, behaviour typically  
associated with receptors and antibodies. In addition,  
antisense peptides have been used as epitopes to generate  
antiidiotypic antibodies (Bost & Blalock *Meth.Enzymol.*,  
178:63 (1989); Araga et al, *PNAS USA*, 90:8747 (1993)).

15 In general, hydrophobic amino acids are complemented by  
hydrophilic amino acids and vice versa, while, on  
average, "uncharged" residues are generally complemented  
by similar residues. This pattern was shown to represent  
the origin of the protein-receptor binding pair  
20 interactions for the related protein, interleukin-2 (Kuo  
& Robb *J.Immunol.*, 137:1538-1543 (1986)), and it has been  
further postulated to result in the relationships that  
typify the immune system. The early work to test the  
validity of this pattern in natural assay systems was  
25 based on high-affinity binding of complements to the  
naturally occurring peptides ACTH (adrenocorticotrophic  
hormone) and  $\gamma$ -endorphin (Bost et al, (1985) *supra*).  
However, to date, much of the work has centred on the  
development of antibody-like molecules and affinity  
30 reagents to probe protein-receptor interaction.

Thus, previous research with the use of such anti-sense  
techniques has centred on their value as affinity agents  
and probes to purify target molecules from complex

mixtures.

We have now found that anti-sense peptides can be designed which exert an antagonistic effect on target ligands by virtue of their ability to bind to complementary "sense" peptide sequences contained therein.

This novel approach will allow the design of novel, potentially effective, therapeutic agents for a whole range of conditions mediated by polypeptides or proteins. Examples of suitable targets are discussed below.

The term interleukin-1 (IL-1) encompasses the two structurally similar and potent cytokines, interleukin-1 $\alpha$  and -1 $\beta$ , which both have a MW of 17kDa, and almost identical tertiary structures despite being the product of two separate genes (Clore et al, *Biochemistry*, 29:5671-5676 (1991); Clore et al, *Biochemistry*, 30:2315 (1991); Driscoll et al, *Biochemistry*, 29:3542-3556 (1990); Finzel et al, *J.Mol.Biol.*, 209:779-791 (1989); Graves et al, *Biochemistry*, 29:2679-2684 (1990); Priestle et al, *EMBO J.*, 7:339-343 (1988); Priestle et al, *Cytokines.Lipocortins*, 349:297-307 (1990). The two forms act, with varying affinities, through the same cell surface receptors (IL-1 RI and IL-1 RII and accessory chain, Dower et al, *Nature*, 324:266-268 (1986); Killian et al, *J.Immunol.*, 136:4509 (1986); Sims & Dower, *J.Biol.Chem.*, 6:112 (1990)) and, with few exceptions, elicit similar responses (Boraschi et al, *Eur.J.Immunol.*, 20:317 (1990); Calkins et al, *Biochem.Biophys.Res.Comm.*, 167:548-553 (1990)).

Through widespread effects, these proteins perform central mediatory roles in immunity, haematopoiesis and the inflammatory response as well as in rheumatoid

5 arthritis and septic shock (Dinarello, *Blood*, 77:1627-1652 (1991); Dinarello and Wolff, *N.Eng.J.Med.*, 328:106 (1993)). Both have been implicated in the amyloid pathology of head trauma associated with Alzheimer's disease (AD) and other neurodegenerative disorders, apparently upregulating the Amyloid Precursor Proteins (APP) (Vandenabeele & Friers, *Immunol.Today*, 12:217 (1991); Buxbaum et al, *PNAS USA*, 89:10075 (1992); Vasilakos et al, *FEBS Lett.*, 354:289 (1994)), a ubiquitous family of transmembrane glycoproteins expressed throughout the body tissues.

15 Tight control of IL-1 activity within biological systems is normally maintained by mechanisms which include a third member of the IL-1 family, namely the naturally occurring N-glycosylated interleukin 1 receptor antagonist (IL-1ra) (Eisenberg et al, *Nature*, 343:341 (1990); Carter et al, *Nature*, 344:633-638 (1990); Hannum et al, *Nature*, 343:336-340 (1990); Seckinger et al, *J.Immunol.*, 139:1541 (1987); Vigers et al, *J.Biol.Chem.*, 269:12874 (1994); Stockman et al, *FEBS Lett.*, 349:79 (1994)), which binds competitively to the IL-1 receptors, but, lacking the necessary trigger domain, fails to elicit any comparable biological response (Dripps et al, *J.Biol.Chem.*, 266:10331-10336 (1991)).

30 In clinical trials, IL-1ra was found to be more effective than traditional treatments for reduction of joint inflammation and discomfort. However, due to its short half-life, and high dosage requirements, it is of little therapeutic value. Potential therapeutic strategies aimed at modulating IL-1 pathological activity have therefore been based on the development and introduction of more stable IL-1ra analogues; site-directed

mutagenesis studies have highlighted several mutations in the IL-1 sequence which give rise to partial antagonism (Ferreira et al, *Nature*, **334**:698-700 (1988)). Other recent attempts to inhibit IL-1 effects include soluble  
5 receptor antagonists and mimics (Bates et al, *Expert Opinion in Therapeutic Patents*, **4**:917 (1994)) as well as pyridinyl-imidazole inhibitors (Lee et al, *Nature*, **372**:739 (1994)).

10 TNF $\alpha$  is widely appreciated as a principal mediator of systemic responses to sepsis and injury (Beutler, B., and Cerami, A., *Ann. Rev. Biochem.*, **57**:505-518 (1988)). Produced by inflammatory cells in response to diverse infectious stimuli and tissue injury, TNF $\alpha$  induces a  
15 cascade of mediators that direct host immunological functions (Fong, et al, *J.Exp. Med.*, **170**:1627-1633 (1989)). While TNF $\alpha$  may thus serve as an essential messenger in host defense, the excessive tissue production of TNF $\alpha$  can mediate detrimental system effects  
20 by acutely precipitating a syndrome similar to that of septic shock (Tracey, et al, *Science* **234**:470-474 (1986)), and lesser degrees of chronic TNF $\alpha$  production appear to induce anorexia and cachexia (Moldawer, et al, *Am. J. Physiol.*, **254**:G450-G456 (1988); Tracey, et al, *J.Exp. Med*  
25 **167**:1211-12278 (1988)). Thus, pathologic conditions may result from the excessive production and activity of TNF $\alpha$ .

The active form of TNF $\alpha$  is believed to be a homotrimer  
30 with 17-kDa subunit polypeptides (Smith., and Baglioni, C., *J. Biol. Chem.*, **262**:6951-6954 (1987)). TNF $\alpha$  and TNF $\beta$ , a related lymphokine, activities are mediated through two distinct receptors, TNFR-p55 and TNFR-p75 (Loetscher, et al, *Cell*, **61**:361-370 (1990)). Both receptors bind TNF $\alpha$

and  $\text{TNF}\beta$  with similar affinities, but they are independently regulated.

5 Eotaxin was recently identified as an important eosinophil chemoattractant detected in bronchoalveolar lavage fluid (BAL) after allergen challenge of sensitised guinea-pigs (Jose et al, *J.Exp.Med*, **179**:881-887 (1994)). The potency of this chemokine has been demonstrated by low dose induction of eosinophil accumulation in guinea-pig 10 airways and skin *in vivo*, and by comparable eosinophil trafficking in guinea-pig and human cells *in vitro* (Jose et al (1994) *supra*; Bousquet.J. and Charez.P., N., *Eng.J.Med*, **323**:1033-1039 (1994)). The role of eosinophil accumulation in Ig-E mediated allergic 15 responses is well known, particularly for asthma (Warringa et al, *J.Allergy Clin.Immunol*, **91**:1198-1205 (1993)), eczema (Leiferman.K.M., *J.Am.Acad.Derm.*, **24**:1101-1112 (1993)), rhinitis (Cantani et al, *J.Invest.Allerg.Clin.Immunol*, **2**:181-186 1992)) and 20 various parasitic infections (Gounni et al, *Am. Rev.Respir.Dis.*, **131**:373-376 1985)). Eotaxin appears to be unique, so far, among the chemokines in its ability to selectively activate eosinophils which accumulate in both guinea-pig skin and airways and is thus a selective 25 subject for inhibition studies.

Interleukin-8 (IL-8) is a CXC class chemokine structurally related to platelet factor 4 (Baggiolini.M., and Clark-Lewis.I., *FEBS*, **307**:97-101 (1992)). It is 30 produced by phagocytes and mesenchymal cells exposed to inflammatory stimuli and activates neutrophils inducing chemotaxis, exocytosis and the respiratory burst (Seitz et al, *J.Clin. Inv.*, **87**:463 (1991)). As a product of different types of cells it can arise in any tissue when



the levels of IL-1 and TNF are enhanced.

Structure-activity relation studies indicate that IL8 binds at the N-terminus (Clark-Lewis et al, *J.Biol.Chem.*, 266:23128 (1991)). No receptor binding or neutrophil activation was observed when the N-terminal sequence Glu-Leu-Arg (ELR) that precedes the first cysteine is deleted. The role of this motif as the main structural determinant of receptor binding is confirmed by site directed mutagenesis studies in which IL-8 mutants, containing alanines in place of the ELR sequence, are all chemotactically inactive (Hebert et al, *J.Biol.Chem.* 266:18989 (1991)).

In a first aspect, therefore, the present invention provides a peptide or polypeptide comprising an amino acid sequence which is antisense to a target peptide or polypeptide sequence, wherein said antisense peptide or polypeptide binds to the target peptide or polypeptide, thereby altering the biological activity of the target peptide or polypeptide or the biological activity of a target molecule which comprises the target peptide or polypeptide.

The term "anti-sense" has heretofore generally been applied to nucleic acid sequences which are capable of binding to complementary nucleic sequences. For example, anti-sense DNA sequences can be generated which in turn can generate mRNA sequences which will bind to mRNA produced from coding/sense strands of DNA, thereby preventing translation. In the context of the present invention, the term "anti-sense peptide or polypeptide" refers to a peptide or polypeptide coded for by a nucleic acid sequence complementary to the nucleic acid sequence coding for the target sequence. An anti-sense peptide or

polypeptide within the context of the present invention also includes a peptide or polypeptide, at least part of whose sequence is anti-sense to a target sequence.

5 Clearly, as the skilled man will appreciate, the concept of targeting a peptide or polypeptide sequence with an antisense peptide or polypeptide can be applied to relatively small biologically active peptides in order to affect their biological activity. Alternatively, the  
10 target sequence will form part of a larger molecule, with the target sequence being involved in the biological activity of the molecule.

15 In one embodiment of this aspect of the invention, the anti-sense peptide or polypeptide can act as an antagonist to or inhibitor of the biological activity of the target sequence or molecule.

20 A preferred group of target molecules are cytokines, for example IL-1 $\alpha$  and/or IL-1 $\beta$ , IL-8 or TNF $\alpha$ . In the case of IL-1 $\alpha$  or IL-1 $\beta$  the anti-sense peptide is preferably anti-sense to a target sequence located within the region of residues 47-55 of IL-1 $\beta$ . Examples of such antisense peptides include:-

25

N-VITFFSL; and  
N-VITFFS.

30 In the case of TNF $\alpha$  the antisense peptide is preferably antisense to a target sequence located within the region of residues 83-91 or 29-34 of TNF $\alpha$ . Examples of suitable peptides include:-

N-DLGLVRDGD;  
N-LGLVRDG; and

N-IGPAVQ.

In the case of IL-8 an example of a suitable antisense peptide is:-

5

N-SKLFS.

Another example of a suitable target molecule for the antisense approach is Eotaxin. Preferably, the antisense peptide is antisense to a target sequence located within  
10 the region 43-49 or 45-50 of Eotaxin. Examples of suitable antisense peptides include:-

N-DILGQFG; and

N-HFVRFD.

15

As discussed above, these antisense peptides could form part of a larger peptide or polypeptide. The key property which any such larger sequence must possess is of course the ability to bind to the target sequence.

20

The skilled man will appreciate that the concept of "anti-sense" peptides or polypeptides can be applied generally to alter the biological properties of a range or "targets". Techniques are readily available, as  
25 discussed in the examples below, for identification of target sequences, which may form part of larger, biologically active, molecules. Once such target sequences have been identified "antisense" peptides or polypeptides can be generated (again using standard  
30 techniques) and tested against the target molecule.

Thus, in a second aspect, the invention provides an anti-sense peptide or polypeptide for use in altering the biological activity of a target sequence or molecule.

In particular, anti-sense peptides or polypeptides would be useful as therapeutic agents by virtue of their ability to alter biological activity of a target sequence or molecule.

5

In a further aspect, therefore, the invention provides an anti-sense peptide or polypeptide for use in medicine, particularly in the treatment or prevention of an inflammatory condition and/or cancer.

10

Generally, the anti-sense peptides or polypeptides of the invention will find application in medicine in the form of a pharmaceutical formulation. In a fourth aspect, therefore, the invention provides the use of an antisense peptide or polypeptide as defined herein in the manufacture of a medicament for use in the prophylaxis or treatment of a condition mediated by a cytokine.

15

In a fifth aspect the invention provides a pharmaceutical formulation comprising at least one anti-sense peptide or polypeptide as defined herein, together with one or more pharmaceutically acceptable carriers, diluents or excipients.

20

In particular embodiments, the target molecule is IL-1 $\alpha$  and/or IL-1 $\beta$ , TNF $\alpha$ , Eotaxin or IL-8 and the anti-sense peptides or polypeptides of the invention can be used to inhibit the biological action of these target molecules and thus find use in treating inflammatory conditions, e.g. septic shock, rheumatic diseases and degenerative arthropathies, including rheumatoid arthritis as well as, in the case of TNF $\alpha$ , cancer.

25

30

In a sixth aspect, the present invention provides a

method of treating a condition mediated by a cytokine which comprises administering to a patient an effective amount of an antisense peptide or polypeptide as defined herein

5

In a final aspect the invention provides a method for the prophylaxis or treatment of an inflammatory condition which comprises administering to a patient an effective amount of an antisense peptide or polypeptide as defined herein, preferably in the form of a pharmaceutical formulation.

10

Preferred features of each aspect of the invention are applicable to each other aspect *mutatis mutandis*.

15

The invention will now be described by way of the following examples, which are not to be construed as in any way limiting the invention.

20

The examples refer to the figures in which:-

FIGURE 1a: shows an alignment of the gene-derived amino acid sequences of IL-1 $\alpha$ , IL-1 $\beta$  and IL-1ra;

25

FIGURE 1b: shows the DNA sequence coding for the  $\beta$ -bulge of IL-1 $\beta$  (Boraschi loop) in alignment with the complementary/antisense DNA sequence;

30

FIGURE 1c: shows a comparison of the Kyte-Doolittle hydropathic profiles of the Boraschi loop sequence, QGEESND (■, antisense peptide, VITFFSL (□) and control peptide (●);

FIGURE 2a: shows the dose dependent inhibition by

antisense peptide VITFFSL of IL-1 $\beta$  stimulated synthesis of serum amyloid A (SAA) (black bars) and haptoglobin (hatched bars) in HuH7 hepatoma cell supernatants after 48h of stimulation;

FIGURE 2b: shows the inhibition of IL-1 $\beta$  interaction with soluble human IL-1 receptor type II (sIL-1 RII) by antisense peptides, VITFFSL (■) and VITFFS (□);

FIGURE 3a: shows surface plasmon resonance (SPR) affinity profiles obtained from the interaction of antisense peptide, VITFFSL, with immobilised IL-1 $\beta$ . Peptide concentrations were 20 $\mu$ M (—), 40 $\mu$ M (- -), 70 $\mu$ M (···), 90 $\mu$ M (-·-), 100 $\mu$ M (-·-·) and 200 $\mu$ M (—);

FIGURE 3b: shows the maximum affinity profile response changes (after background correction) plotted as a function of VITFFSL concentration. Results were obtained with immobilised IL-1 $\beta$  (surface concentration 22 $\mu$ g/ml) in the absence ( $\Delta$ ) and presence (O) of sense peptide QGEESND (equimolar with VITFFSL), and immobilised IL-1 $\alpha$  ( $\Delta$ ) (surface concentration 15 $\mu$ g/ml);

FIGURE 4: shows a comparison of the hydropathic profiles of TNF $\alpha$  residues 83-91 and the corresponding antisense peptide;

FIGURE 5: shows a comparison of the hydropathic profiles of the antisense peptide for region 83-91 of TNF $\alpha$  and the region 91-99 of TNFR 55;

FIGURE 6: shows a comparison of the hydropathic

profiles of TNF $\alpha$  residues 83-91, TNFRp55 (91-99) and TNFRp75 (91-99);

5           FIGURE 7:       shows the results of TNF inhibition assays using two antisense peptides;

FIGURE 8:       shows a comparison of the average hydrophathy of huMCP-1 and gp EOTXAIN;

10          FIGURE 9:       shows a comparison of the average hydrophathy of hEOTAXIN and hMCP-1;

15          FIGURE 10:    shows a comparison of the hydrophathic profiles of gp EOTAXIN (45-50), a corresponding antisense peptide (HFVRFD) and CCKR3 receptor fragment 146-152;

20          FIGURE 11:    shows a comparison of the hydrophathic profiles of a portion of the sequence of IL-8 (AKELR), a corresponding antisense peptide (SKLFS) and IL-8R sequence (AKFLT).

#### EXAMPLE 1: IL-1

##### Standard Methods

##### 25    **Peptide generation**

30    All peptides were generated to a standard Fmoc protocol using a fully automated Applied Biosystems 431A synthesizer, software version 1.1 using Fmoc protected amino acids acquired from Bachem (UK) Ltd and the Rink Amide MBHA resin purchased from Novabiochem.

##### **Peptide purification.**

Freeze dried peptides were desalted on P2 Biogel gel filtration column (2 cm x 30 cm) eluted with 0.1% TFA

then loaded onto a Pharmacia biotech. Pep RPC HR10/10 column and eluted at 1.5 ml/min on linear gradient from 0.1% TFA<sub>(aq)</sub> to 100% acetonitrile, 0.1% TFA, and their identity verified by +ve FABMS.

5

The first stage in the design antisense peptide inhibitors was to identify a suitable target region, from amongst the overlapping functional regions of IL-1 $\alpha$  and IL-1 $\beta$ , against which antisense peptides could be designed to act. Therefore, the X-ray crystal structure of IL-1 $\beta$  (Finzel et al, (1989) *supra*; Priestle et al, (1988), *supra*), IL-1 $\alpha$  (Graves et al, (1990), *supra*) and IL-1ra (Vigers et al, (1994), *supra*) were reviewed in conjunction with the results of recent mutational studies performed on all three proteins (Evans et al, *J.Biol.Chem.*, **270**:11477 (1995); Labriola-Tompkins et al, *Prot.Eng.*, **6**:535 (1993); Labriola-Tompkins et al, *PNAS USA*, **88**:11182 (1991); Grutter et al, *Prot.Eng.*, **7**:663 (1994); Gayle et al, *J.Biol.Chem.*, **268**:22105 (1993); Kawashima et al, *Prot.Eng.*, **5**:171 (1992)).

20

When an overlay of all three structures was made it was observed that both IL-1 $\beta$  and IL-1 $\alpha$  possess a  $\beta$ -bulge structure, proximal to the known receptor binding amino acid residues, which is absent in the antagonist IL-1ra. This appeared to represent the only significant difference between the three-dimensional structures of IL-1ra and the other two IL-1 isoforms. This absence of secondary structure was also mirrored at the primary amino acid sequence level, where IL-1ra was found to have no equivalent stretch of amino acids to the  $\beta$ -bulge regions of IL-1 $\beta$  (residues 48-54[mature protein sequence]) or IL-1 $\alpha$  (residues 60-66[mature protein sequence]) (fig 1a).

25

30



These structural differences suggested that the  $\beta$ -bulge regions of IL-1 $\beta$  and IL-1 $\alpha$  might be promising targets for antisense peptide inhibitors. We were confirmed in this approach firstly by the suggestion (Auron et al, *Biochem.*, 31:6632 (1992)) that these same  $\beta$ -bulge regions may act as the "early trigger" for IL-1 receptor mediated gene transcription, and secondly by reports from Boraschi and coworkers (Antoni et al, *J.Immunol.*, 137:3201 (1986); Boraschi et al, *J.Exp.Med.*, 168:675 (1988)) that a peptide, corresponding in sequence to the  $\beta$ -bulge region IL-1 $\beta$  (residues 47-55 [mature protein]), possesses partial IL-1 agonist activity.

the  $\beta$ -bulge of IL-1 $\beta$  is more pronounced than that of IL-1 $\alpha$  (fig 1a) and therefore it was anticipated that  $\beta$ -bulge directed inhibitors might be more effective against IL-1 $\beta$  than IL-1 $\alpha$ . As a result, antisense peptides were designed to primarily target the  $\beta$ -bulge region of IL-1 $\beta$ , which was re-christened the Boraschi loop.

Antisense peptides to the Boraschi loop were designed with reference to the DNA sequence of IL-1 $\beta$  (March et al, *Nature*, 315:641 (1985)). Having identified the DNA sequence coding for the loop, the complementary/antisense DNA sequence was deduced and the code translated in the 5'→3' direction (fig 1b). Two antisense peptides were then synthesised (see above) on the basis of the antisense code. The first with the sequence VITFFS, complementary to Boraschi-loop segment GEESND (IL-1 $\beta$  residues 49-54), and the second with the sequence VITFFSL, complementary to the Boraschi-loop segment QGEESND (IL-1 $\beta$  residues 48-54). Both peptides were hydrophobic and were therefore prepared as C-terminal amide derivatives to aid aqueous solubility. The Kyte-

Doolittle hydropathic profile of VITFFSL plotted (fig 1c) with the profile of QGEESND illustrates the mutual complementarity of their hydropathic profiles. The hydropathic profile of VITFFSL was also found as well to be reasonably complementary to the profile of the IL-1 $\alpha$   $\beta$ -bulge sequence, KSSKDDA (residues 60-66), leading us to believe that VITFFSL and VITFFS may also target IL-1 $\alpha$ , even though these peptides had not been primarily designed to do this. Therefore tests were carried out to try to inhibit the effects of both IL-1 $\alpha$  and IL-1 $\beta$ .

The antisense peptides were tested for biological effect using an HuH7 hepatoma cell line assay system (Bevan & Raynes, *J.Immunol*, **147**:2574 (1991)). In this assay system serum amyloid A (SAA) and haptoglobin are induced directly in response to IL-1 (Raynes et al, *Clin.Exp.Immunol.*, **83**:448 (1991)). Antisense peptide was predissolved in DMSO (10mg/ml) and diluted, to various concentrations (see fig 2a), in the wells of 24-well plates which contained confluent HuH7 cells under the stimulation of IL-1 $\beta$  (1ng/ml). Specific protein concentrations were measured by ELISA.

Antisense peptides, VITFFSL and VITFFS, were found to inhibit both IL-1 $\beta$  and IL-1 $\alpha$  stimulated synthesis of SAA and haptoglobin in a dose dependent manner (fig2a; table 1). The levels of inhibition are approaching or even exceeding those observed when either IL-1ra or soluble human IL-1 type II receptor (sIL-1 RII) were used as inhibitors in the same assay system (table 1). The data show (fig 2a: table 1) that SAA was inhibited more readily than haptoglobin consistent with previous observations with IL-1ra (Bevan & Raynes (1991), *supra*).

Table 1

PEPTIDE	%SAA inhibition		% Haptoglobin inhibition	
	IL-1 $\alpha$ *	IL-1 $\beta$ *	IL-1 $\alpha$ *	IL-1 $\beta$ *
N-VITFFSL'	75	78	ND	66
N-VITFFS'	55	65	ND	57
IL-1ra'	100	100	100	100
sIL-1 RII'	25	90	38	75
N-VFITSFL'	<10	<10	<10	<10
N-LSFFTIV'	<10	<10	<10	<10
N-LLSLLPV'	<10	<10	<10	<10
N-LLSLLRV'	<10	<10	<10	<10

\* 1ng/ml

† 20 $\mu$ g/ml‡ 10 $\mu$ g/ml§ 10 $\mu$ g/ml (Bevan & Raynes, (1991), *supra*)

In order to test the specificity of IL-1 inhibition by the two antisense peptides, four more peptides were synthesised as controls and tested in the assay (table 1). The choice of controls was dictated by the apparent importance of hydropathic complementarity in determining physical interaction between sense and antisense peptides (Shai et al, *Biochem*, 28:8804 (1989); Shai et al,

*Biochem*, 26:669 (1987)). This suggested that reordering the amino acid sequence of the antisense peptide, to alter the hydropathic profile, should abolish the interaction with the sense peptide, whilst by contrast, a peptide of similar hydropathic profile to the antisense peptide, but different amino acid sequence, would be expected to interact with the original sense peptide.

In keeping with the first part of this analysis, VFITSFL (a reordered peptide with altered hydropathic profile; fig 1d) failed to measurably inhibit IL-1. However, LSFFTIV (the reverse peptide with an identical profile) and LLSLLRV (a peptide with similar profile but different sequence) also failed to inhibit IL-1 in contradiction to the second part of the analysis. Finally, there was some precedent (Bost et al (1985), *supra*) to indicate that a peptide whose sequence was derived by translating the antisense codon in the 3'→5' direction (as opposed to the 5'→3' direction used above) should interact with a sense peptide. However, the corresponding peptide LLSLLPV was also found not to inhibit IL-1. Therefore, the inhibition of IL-1 by VITFFSL and VITFFS appeared very specific.

The origin of this specificity was analysed with the aid of a surface plasmon resonance (SPR) biosensor which was used to determine if antisense peptide VITFFSL was directly binding to IL-1 $\beta$  and IL-1 $\alpha$ . Both cytokines were immobilised on SPR cuvettes and treated with increasing concentrations of VITFFSL under conditions comparable to the HuH7 assay. In both cases, a significant binding interaction was observed (fig 3). Analysis of the affinity profiles revealed that dissociation constants ( $K_d$ ) (table 2) for the interaction with both cytokines were comparable to those measured for the interaction of

other sense and antisense peptides (Shai et al, (1989),  
*supra*). Moreover, the stoichiometry of association was  
calculated to be 4-5 peptides per immobilised cytokine  
molecule, a figure which also agrees with previous  
peptide/antisense peptide studies (Shai et al, (1989),  
*supra*). In order to investigate the specificity of the  
binding interaction, six alternative proteins (IL-1ra,  
interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-8  
(IL-8), lysozyme and the glycoprotein CD59) were  
immobilised on SPR cuvettes and treated with VITFFSL in  
the same way. VITFFSL did not interact significantly with  
any of these six control proteins suggesting that the  
binding interaction of VITFFSL with IL-1 $\beta$  and IL-1 $\alpha$  was  
specific.

**Table 2**

Peptide		$k_{\text{ass}} (\text{M}^{-1}\text{s}^{-1})$	$10^4 \times K_{\text{diss}} (\text{s}^{-1})$	$K_{\text{d}} (\mu\text{M})$
VITFFSL	IL-1 $\alpha$	56.3	6.5	11.4
	IL-1 $\beta$	64.5	6.6	10.2
LSFFTIV	IL-1 $\alpha$	88.9	249.9	281.1
	IL-1 $\beta$	71.1	113.7	160.0
VFITSFL	IL-1 $\alpha$	*	*	*
	IL-1 $\beta$	*	*	*
LLSLLRV	IL-1 $\alpha$	60.8	210.8	345.6
	IL-1 $\beta$	86.5	252.4	291.3
LLSLLPV	IL-1 $\alpha$	52.4	318.5	607.7
	IL-1 $\beta$	48.4	203.8	419.9

\* Too weak to be determined

The interaction of the four control peptides (VFITSFL, LSFFTIV, LLSLLRV and LLSLLPV) with IL-1 $\beta$  and IL-1 $\alpha$  was also investigated with the SPR biosensor. The data obtained (table 2) appears to offer an explanation for the specificity of peptides VITFFSL and VITFFS as IL-1 inhibitors. In keeping with the importance of hydrophobic complementarity in antisense/sense peptide interactions, the reordered peptide VFITSFL was found not to interact with either IL-1 $\beta$  or IL-1 $\alpha$ . The other three peptides, whose hydrophobic profiles are similar to VITFFSL, did interact with both cytokines but at least an order of magnitude more weakly. The association rates,  $k_{\text{ass}}$ , of these three peptides and VITFFSL with the cytokines were the same within experimental error. Therefore, the tighter binding shown by VITFFSL resulted from the slower dissociation rate of this peptide. Presumably, the failure of peptides LSFFTIV, LLSLLRV and LLSLLPV to show inhibition of IL-1 within the experimental limits of the HuH7 assay (<10% inhibition) is a reflection of their weaker associations (between 20 and 60-fold worse) with both IL-1 $\beta$  and IL-1 $\alpha$ .

Having established a specific association between VITFFSL and both IL-1 $\beta$  and IL-1 $\alpha$ , a receptor binding assay was then carried out to establish if VITFFSL and VITFFS could also inhibit the interaction of IL-1 with receptor. Accordingly, immobilised IL-1 $\beta$  was treated with soluble receptor sIL-1 RII in the presence and absence of both peptides (fig 2b). IL-1 $\beta$  was immobilised (4°C, 16h) on Immulon II plates in PBS buffer. The plates were blocked with PBS tween-20 (0.05%, v/v) containing BSA (1%, w/v) followed by the addition of sIL-1 RII (4ng/ml) in the presence of peptide at various concentrations (see fig 2b). In the event, both peptides were able to inhibit

receptor binding, the former by at least 63% compared to control binding in the absence of peptide.

5 The results of all the experiments described above, interlock to support the view that antisense peptides VITFFSL and VITFFS are specific inhibitors of IL-1 stimulated protein synthesis whose mechanism of action probably involves direct association with the cytokine thereby blocking receptor binding. Therefore, both  
10 peptides do appear to be acting as "mini receptor" inhibitors. The evidence suggests that the peptides are binding to the  $\beta$ -bulge structures which the cytokines possess. Firstly, the interaction of VITFFSL with IL-1 $\beta$  was severely reduced in the presence of the sense peptide QGEESND, presumably because the sense peptide is competitive with IL-1 $\beta$ . Secondly, VITFFSL was unable to  
15 interact with IL-1 $\alpha$  (see above) which lacks the  $\beta$ -bulge structure (fig 1). Finally, both VITFFSL and VITFFS were found to be weak inhibitors of the association between  
20 IL-1 $\beta$  and the low affinity antibody BhrD2 which is specific to IL-1 $\beta$  amino acid residues 45-87 (incorporating the Boraschi loop structure).

#### EXAMPLE 2:TNF $\alpha$

##### Standard Methods

##### **Peptide generation**

Peptides were generated to a standard Fmoc protocol using (i) a fully automated Applied Biosystems 431A synthesizer, software version 1.1 using Fmoc protected  
30 amino acids acquired from Bachem (UK) Ltd. and Rink Amide MBHA resin purchased from Novabiochem, and (ii) Shimadzu RF SPPS automated synthesizer.

**Peptide purification**

Freeze dried peptides were desalted on a P2 Biogel gel filtration column (2cm x 30cm) eluted with 0.1% TFA, 20% Acetonitrile (ACN) peptides were further purified by loading onto a Pharmacia biotech. Pep RPC HR 10/10 column and eluted at 1.0 ml/min on a linear gradient from 0.1%TFA, 20% ACN to 0.1%TFA, 100% ACN. Their identity is verified by +ve FABMS.

**Testing on Iasys system**

Test and control proteins were immobilised onto carboxymethyl dextran (CMD) coated cuvette surfaces at 37°C and pH 7.4, in PBS buffer using a standard NHS/EDC coupling protocol (described in the Iasys manual). Coupling times of 30-40 mins were allowed to generate a surface concentration suitable for analyte assay runs.

**High pressure affinity chromatography(HPAC)**

Peptides were attached to a Pharmacia activated CH sepharose 4B employing standard NHS coupling methods and packed on a CR10/10 column. Ligand binding is assessed by both zonal and continuous elution methods (Shai et al, *Biochemistry*, 26:669-675 (1987)).

Site directed mutagenesis studies on TNF $\alpha$  have been carried out with a view to define the receptor binding site structural-functional relationship (Zhang et al, *J. Biol. Chem.*, 267:24069-24075 (1992)). It appears that trimer formation is necessary for receptor binding and thus cytotoxic activity. Several TNF $\alpha$  surface mutants are found to allow trimer formation but impair cytotoxic activity, presumably through a lack of receptor activation. From these studies, four regions of TNF $\alpha$  appear critical for in vitro receptor biological



activity. Of these, surface residues 83-91 represent the most solvent exposed region, as viewed from the X-ray crystal structure with a Quanta Molecular Graphics program. A single point mutation of Tyr<sup>87</sup> to any other residue identity impairs all biological activity. On this basis, antisense peptides (aFNT I and II) were designed to bind to this section of protein as shown below.

TNF $\alpha$  seq.83-91

10	N	Ile	Ala	Val	Ser	Tyr	Gln	Thr	Lys	Val	C
		I	A	V	S	Y	Q	T	K	V	
		A T C	G C C	G T C	T C G	T A C	C A G	A C C	A A G	G T C	
		T A G	C G G	C A G	A G C	A T G	G T C	T G G	T T C	C A G	
		D	G	D	R	V	L	G	L	D	
15	C	Asp	Gly	Asp	Arg	Val	Leu	Gly	Leu	Asp	N

aFNT I      NH<sub>2</sub>   D L G L V R D G D   COOH

aFNT II     NH<sub>2</sub>   L G L V R D G   COOH

Another of the TNF $\alpha$  solvent exposed active regions (29-34) is known to be critical in binding to the p75 receptor only. A complementary peptide to this region could specifically prevent p75 activation and thus allow the local cytotoxic TNF response mediated by the TNFRp55. This could provide the therapeutic basis for administering high doses of TNF $\alpha$  in anti-cancer therapy without the systemic toxicity dependent on p75 activation (Van Ostade et al, Nature, 361:266-269 (1993)). An antisense peptide designed to bind to this region is shown below.

TNF $\alpha$  seq. 29-34

N	Leu	Asn	Arg	Arg	Ala	Asn	C
	L	N	R	R	A	N	
	C T G	A A C	C G C	C G G	G C C	A A T	
5	G A C	T T G	G C G	G C C	C G G	T T A	
	Q	V	A	P	G	I	
C	Gln	Val	Ala	Pro	Gly	Ile	N

aFNT III    NH<sub>2</sub>   I G P A V Q   COOH

10

15

20

25

The Molecular Recognition theory (MRT) theory purports an idea that antisense relationships form the basis of receptor-effector recognition. This supposition was tested theoretically for TNF $\alpha$  : if recognition between receptors and effectors is based on antisense, then an antisense peptide designed against a known activating region of TNF $\alpha$  might share some amino acid homology with conserved sections of the p55 and p75 receptors. An antisense homology search for TNF 83-91 revealed this to be the case: a 5 residue section of the nine residue effector sequence shared homology (including conservative substitutions) with a section of the p55 and p75 receptors. Moreover, the hydropathy plots for these receptor segments and for the antisense peptides aFNT were very similar and display a strong inverse correlation with the hydropathy trace for TNF 83-91 (figs 4,5,6).

30

35

Peptides were assayed for TNF $\alpha$  inhibitory activity using the L929 cytotoxicity assay. L929 cells are trypsinized, washed and resuspended at  $8 \times 10^5$  cells/ml and 50 $\mu$ l added to wells of a 96 well plate. TNF $\alpha$  standards of 30, 10, 3, 1 0.3 and 0.1 U/ml were added and either 7.5 or 3.75 U/ml of TNF incubated with various concentrations of peptide for less than 30 min. before adding to the cells.

Actinomycin D was added to a final concentration of 1µg/ml to increase sensitivity. The plates were incubated for 24h, MTT was added and left for 4h before removal of the supernatant and colour determination, by dissolving in isopropanol:1-propanol (1:9 vol; 100µl) and measuring at 550nm. The results are shown in figure 7.

### EXAMPLE 3:EOTAXIN

Standard methods were as for example 2.

The cDNA cloning and expression of eotaxin revealed interesting relationships between sequence homology and respective selectivities of other CC chemokines. It shared the greatest homology (53%) with human monocyte chemoattractant protein 1 (MCP1), a monocyte chemotaxant but not an eosinophil attractant in guinea-pig or human assay (Jose et al, (1994) *supra*). Less homolgy is observed for the human macrophage inflammatory protein (hMIP-1α, 31%) and hRANTES (26%) which are both inactive to eosinophils in guinea-pig skin but are active in human *in vitro* studies (Jose et al, (1994) *supra*). Based on previous experiments showing that complementary peptides can bind to one another and that this interaction is associated with an inverse correlation in hydropathy plot (according to the Kyte and Doolittle scale (Fassina et al, *Int.J.Peptide.Res*, 39:549-556 (1992)), it was theorised that a comparison of both hydropathy plots and structural homology might yield some information on possible effector regions of these molecules. The analysis of average hydropathy plots for MCP1, gpEotaxin and huEotaxin revealed a striking similarity apart from three distinct regions (fig.8,9) . One of these regions on MCP1 correlated to a very solvent exposed 'loop' as viewed from the X-ray crystal structure.

Homology models of both gp and hu Eotaxin were constructed based on the Brookhaven Protein Databank file coordinates of MCP1 and the disparate region of hydropathic profile corresponded with the same solvent exposed loop (fig. 8). As MCP1 lacks eosinophil stimulatory activity in both guinea-pig and human assays, despite showing high sequence and structural homology to eotaxin, it was hypothesised that this loop sequence (45-49) in both hu and gp eotaxin are possible activation regions and further that their complementary peptides (see below) might bind to them and thus inhibit their biological activity.

#### hu EOTAXIN 43-49 and antisense sequence derivation

15	sense sequence	NH <sub>2</sub>	Thr	Lys	Leu	Ala	Lys	Asp	Ile	
	COOH									
	cDNA sequence		A C C	A A A	C T G	G C C	A A G	G A T	A T C	
			T G G	T T T	G A C	C G G	T T C	C T A	T A G	
			G	F	Q	G	L	I	D	
20	antisense seq.	COOH	Gly	Phe	Gln	Gly	Leu	Ile	Asp	
	NH <sub>2</sub>									

NH<sub>2</sub> I L G Q F G COOH antisense to hu EOTAXIN 43-48

NH<sub>2</sub> D I L G Q F COOH antisense to hu EOTAXIN 44-49

#### gp EOTAXIN 45-50 and antisense sequence derivation

	sense sequence	NH <sub>2</sub>	Ile	Lys	Pro	Asp	Lys	Met	COOH
			I	K	P	D	k	M	
	cDNA sequence		A T C	A A A	C C T	G A C	C A A	A T G	
30			T A G	T T T	G G A	C T G	G T T	T A C	
			D	F	R	V	F	H	
	antisense seq.	COOH	Asp	Phe	Arg	Val	Phe	His	NH <sub>2</sub>

NH<sub>2</sub> H F V R F D COOH antisense to gp EOTAXIN 45-50

The receptors through which many of the CC chemokines

elicit responses have been cloned and expressed. Through cross-desensitization experiments, it was proposed that eosinophils have, among others, a shared receptor for eotaxin, CC CKR3 (Combadiere et al, *J.Biol.Chem.*, 270;27:16941-16949 (1995)). Based on the concept that inverse hydropathy characterised possible binding complements, it seems reasonable that an eotaxin receptor would include a region antisense to the putative effector region on eotaxin. This indeed was the case: a sequence of four residues were directly antisense to the putative activating loop on gp Eotaxin, whilst the two flanking residues were hydropathically complementary to the residues expected to be proximal when aligned N to C and C to N respectively (fig. 10 displays hydropathy plots and residue identity).

#### EXAMPLE 4:IL-8

Standard methods were as for example 2.

IL-8 in humans is known to elicit its response through two distinct seven transmembrane spanning receptors, IL-8R1 and IL-8R2. In accordance with the complementary approach outlined above, it was theorised that if a region of one of the known receptors was homologous to the antisense peptide sequence of the IL-8 activating region, then a synthetic equivalent of that sequence may inhibit IL8 activity by binding to it. A search for homology between the ELR antisense complementary sequences and the receptor sequences revealed several segments satisfying the antisense combinations; of these one was integral to a 5 residue sequence whose hydropathy profile displays excellent negative correlation to the AKELR IL-8 N terminal region (fig. 11).

CLAIMS

1. A peptide or polypeptide comprising an amino acid sequence which is antisense to a target peptide or polypeptide sequence, wherein said antisense peptide or polypeptide binds to the target peptide or polypeptide, thereby altering the biological activity of the target peptide or polypeptide or the biological activity of a target molecule which comprises the target peptide or polypeptide.
2. An antisense peptide or polypeptide as claimed in claim 1, which is wholly antisense for a target sequence.
3. An antisense peptide or polypeptide as claimed in claim 1 or claim 2, which binds to a target sequence which forms part of a larger target molecule.
4. An antisense peptide or polypeptide as claimed in any one of claims 1 to 3 which acts as an antagonist for or inhibitor of the target sequence or molecule.
5. An antisense peptide or polypeptide as claimed in any one of claims 1 to 4, wherein the target molecule is a cytokine.
6. An antisense peptide or polypeptide as claimed in claim 5, wherein the cytokine is IL-1 $\alpha$  and/or IL-1 $\beta$ , TNF $\alpha$  or IL-8.
7. An antisense peptide or polypeptide as claimed in claim 6, which is antisense to a sequence within the region of residues 47-55 of IL-1 $\beta$ .

8. An antisense peptide or polypeptide as claimed in claim 7 which includes the amino acid sequence:

N-VITFFSL; or  
N-VITFFS.

9. An antisense peptide or polypeptide as claimed in claim 6, which is antisense to a sequence within the region of residues 83-91 or 29-34 of  $\text{TNF}\alpha$ .

10. An antisense peptide or polypeptide as claimed in claim 9 which includes the amino acid sequence:

N-DLGLVRDGD;  
N-LGLVRDG; or  
N-IGPAVQ.

11. An antisense peptide or polypeptide as claimed in claim 6 wherein the target molecule is IL-8 and the peptide or polypeptide includes the amino acid sequence:  
N-SKLFS.

12. An antisense peptide or polypeptide as claimed in any one of claims 1 to 4 wherein the target molecule is EOTAXIN.

13. An antisense peptide or polypeptide as claimed in claim 12 which include the amino acid sequence:

N-DILGQFG; or  
N-HFVRFD.

14. An antisense peptide or polypeptide for use in altering the biological activity of a target sequence or molecule.

15. An antisense peptide or polypeptide for use in medicine.

5 16. An antisense peptide or polypeptide as claimed in claim 14 or claim 15, modified by any one or more of the features of claims 2 to 13.

10 17. An antisense peptide or polypeptide as claimed in claim 16 for use in treating or preventing an inflammatory condition.

15 18. The use of an antisense peptide or polypeptide as defined in any one of claims 1 to 13 in the manufacture of a medicament for use in the prophylaxis or treatment of a condition mediated by a cytokine.

19. The use as claimed in claim 18 wherein the cytokine is IL-1 $\alpha$  or IL-1 $\beta$ , TNF $\alpha$  or IL-8.

20 20. The use as claimed in claim 19 wherein the condition is an inflammatory condition.

25 21. The use as claimed in claim 20 wherein the inflammatory condition is rheumatoid arthritis or septic shock.

22. The use as claimed in claim 19 wherein the cytokine is TNF $\alpha$  and the condition is cancer.

30 23. A pharmaceutical formulation comprising at least one antisense peptide or polypeptide, together with one or more pharmaceutically acceptable carriers, diluent or excipient.



24. A pharmaceutical formulation as claimed in claim 23, wherein the antisense peptide or polypeptide is as defined in any one of claims 1 to 13.

5 25. A method of treating a condition mediated by a cytokine which comprises administering to a patient an effective amount of an antisense peptide or polypeptide.

10 26. A method as claimed in claim 25 wherein the antisense peptide or polypeptide is as defined in any one of claims 2 to 13.

15 27. A method for the prophylaxis or treatment of an inflammatory condition which comprises administering to a patient an effective amount of an antisense peptide or polypeptide as defined in any one of claims 5 to 13.

20 28. A method for the treatment of cancer which comprises administering to a patient an effective amount of an antisense peptide or polypeptide as defined in claim 9 or claim 10.

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		N		C
IL-1 $\alpha$	53	K F D M G A Y K S S K D D A K I .. T V I		
IL-1 $\beta$	41	V F S M S F V <i>Q G E E S N D</i> K I .. P V A		
IL-1 $\alpha$	45	K I .. .. D V V .. .. .. .. P I E P H A		

FIG. 1A

*Q G E E S N D*  
 5' CAA GGA GAA GAA AGT AAT GAC 3' (IL-1 $\beta$  code)

3' GTT CCT CTT CTT TCA TTA CTG 5' (Antisense code)  
*L S F F T I V*

FIG. 1B

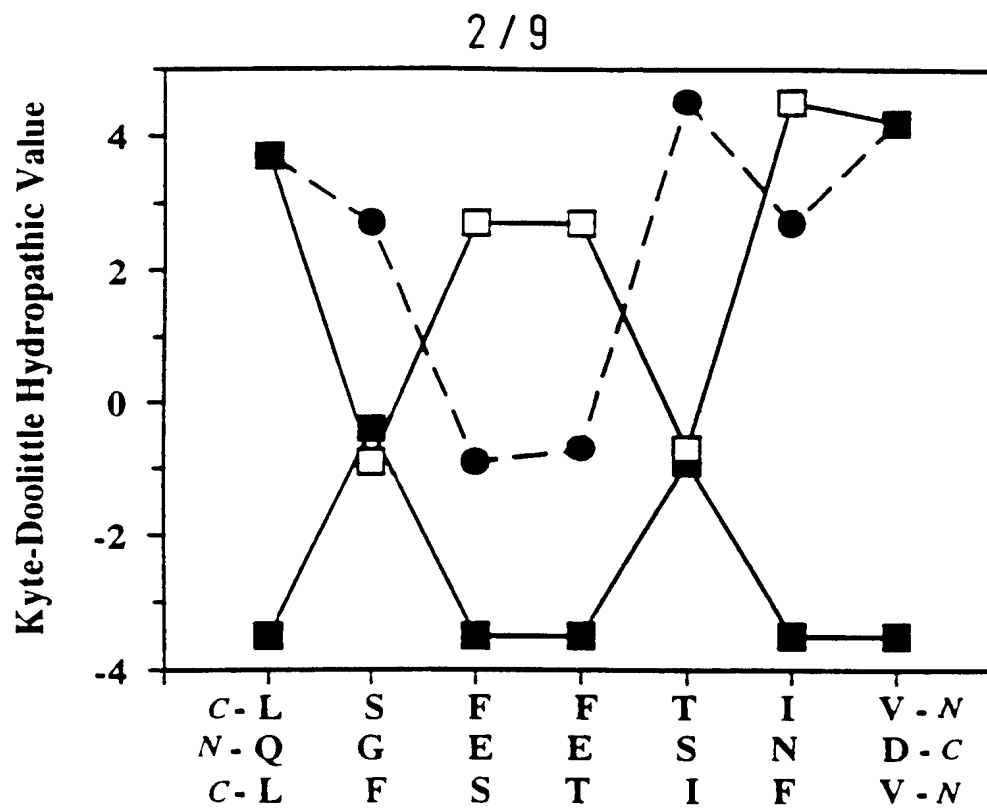


FIG. 1C

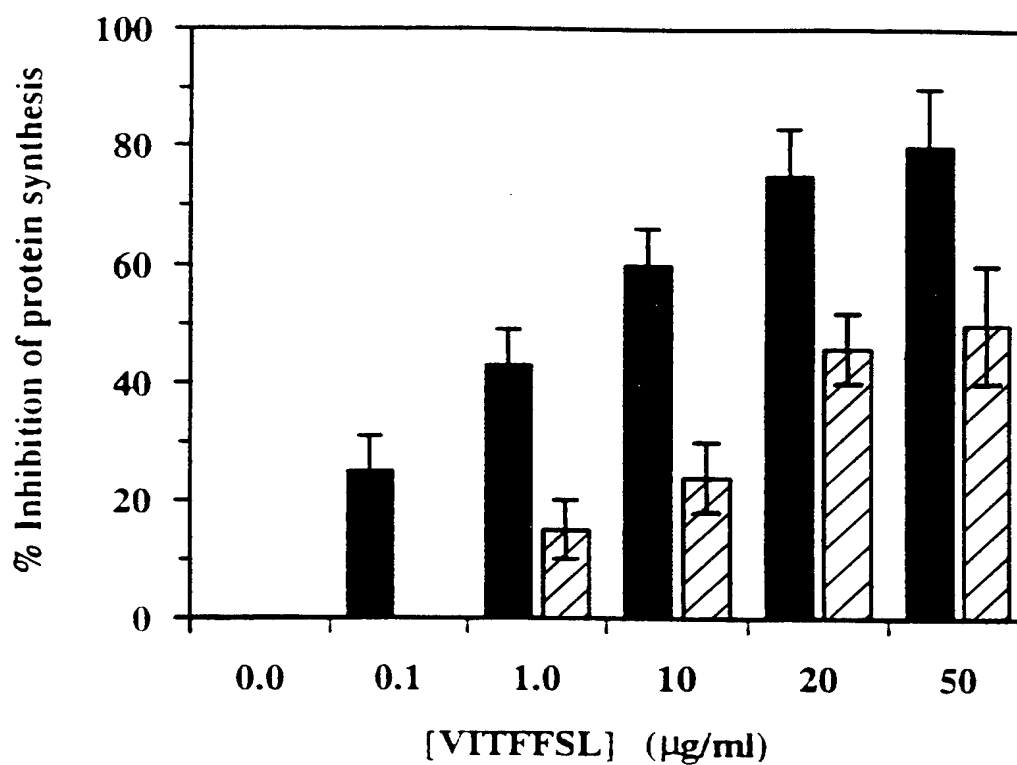


FIG. 2A

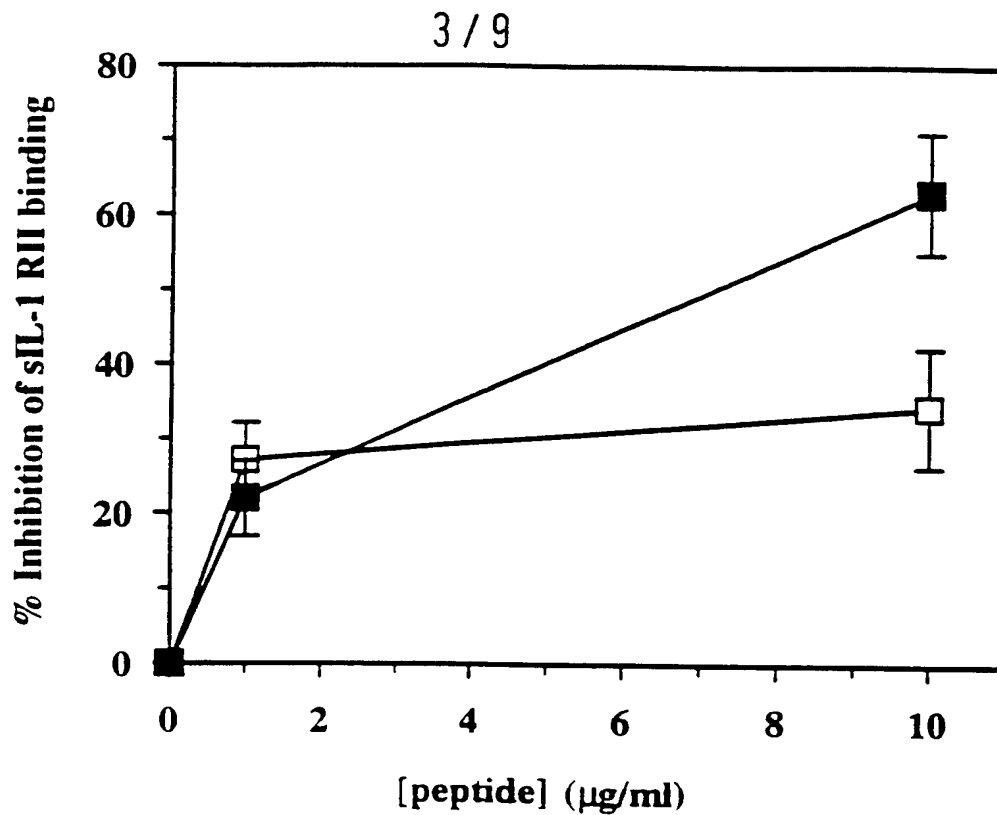


FIG. 2B

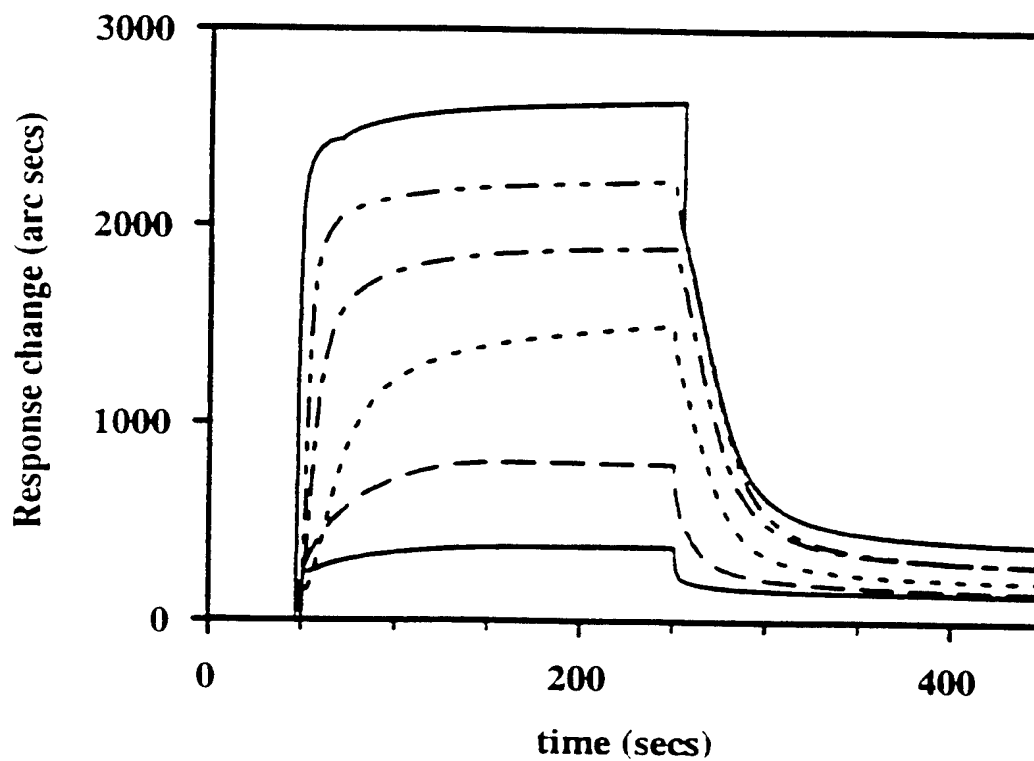


FIG. 3A

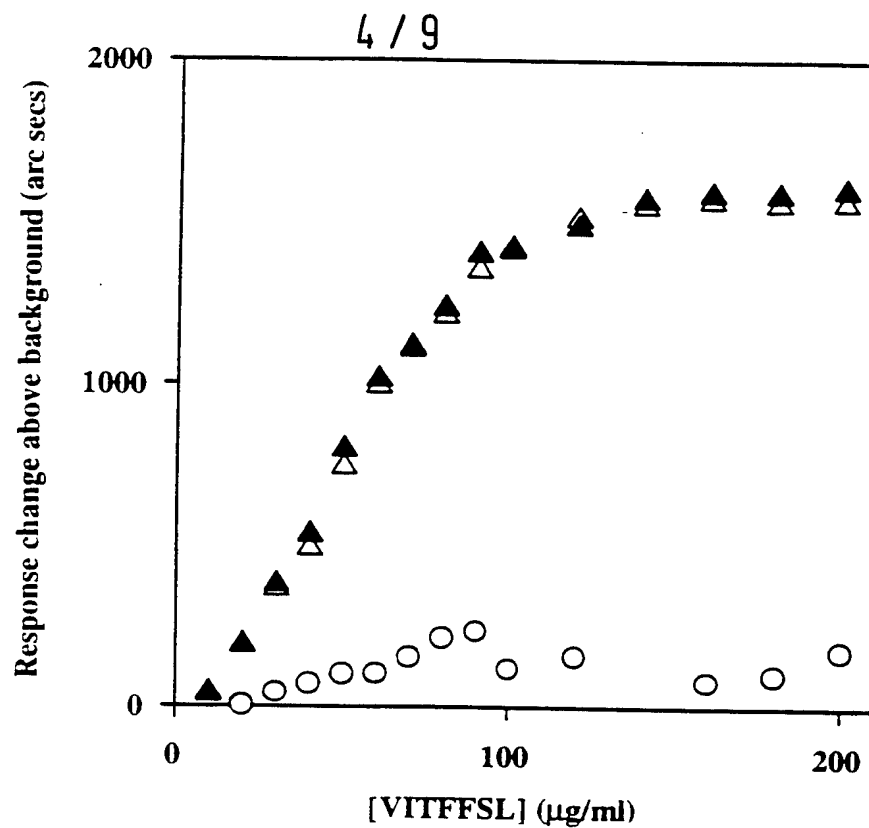
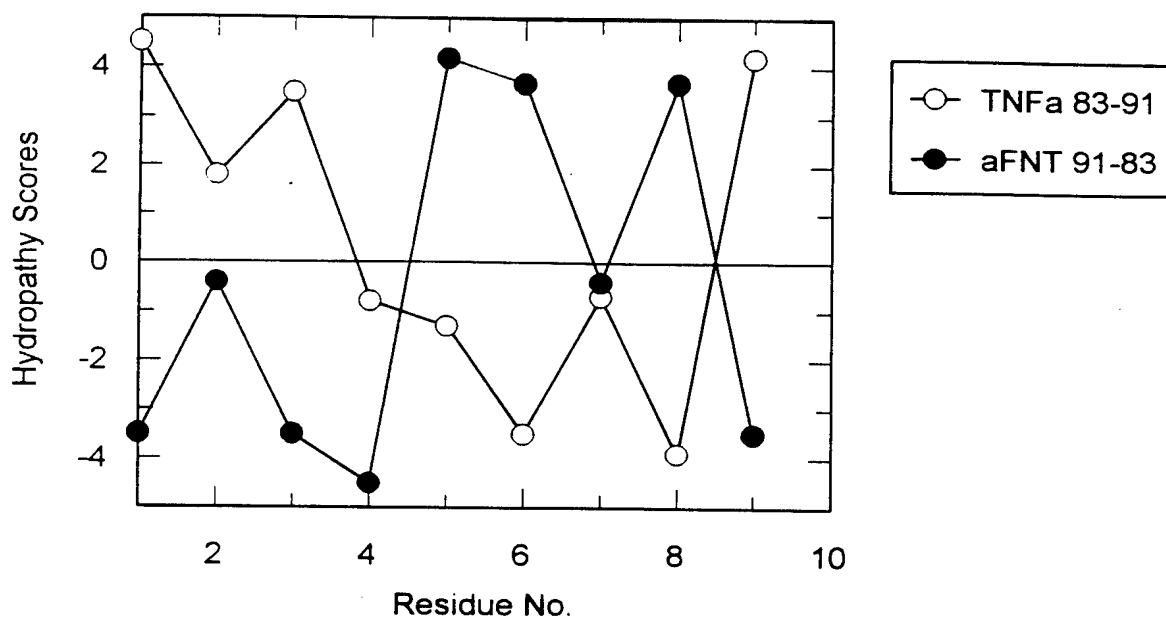
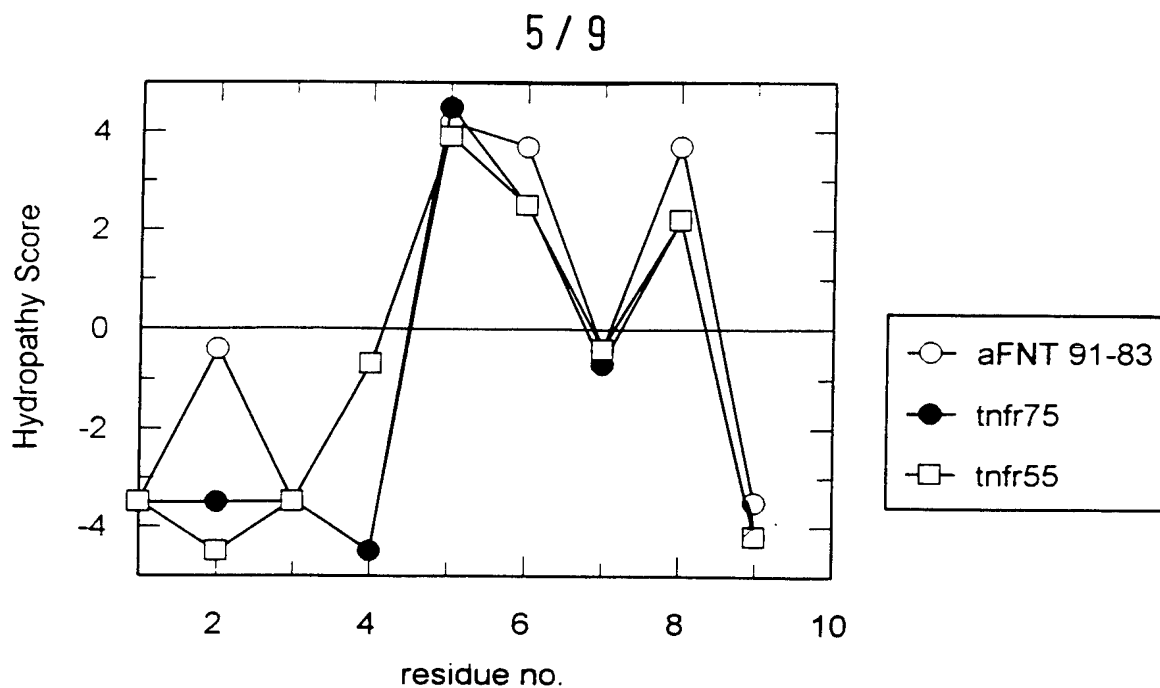


FIG. 3B



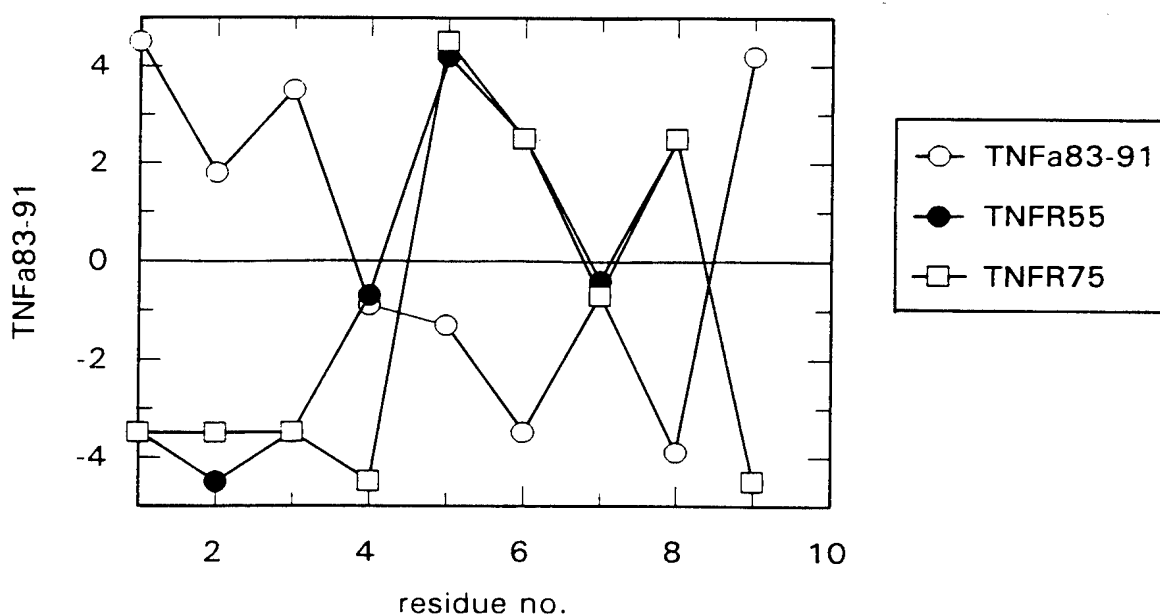
I A V S Y Q T K V TNFα  
D L G L V R D G D aFNT

FIG. 4



D G D R V L G L D aFNT  
 D R D T V C G C R TNFR p55 (91-99)  
 E Q N R I C T C R TNFR p75  
 (91-99)

FIG. 5

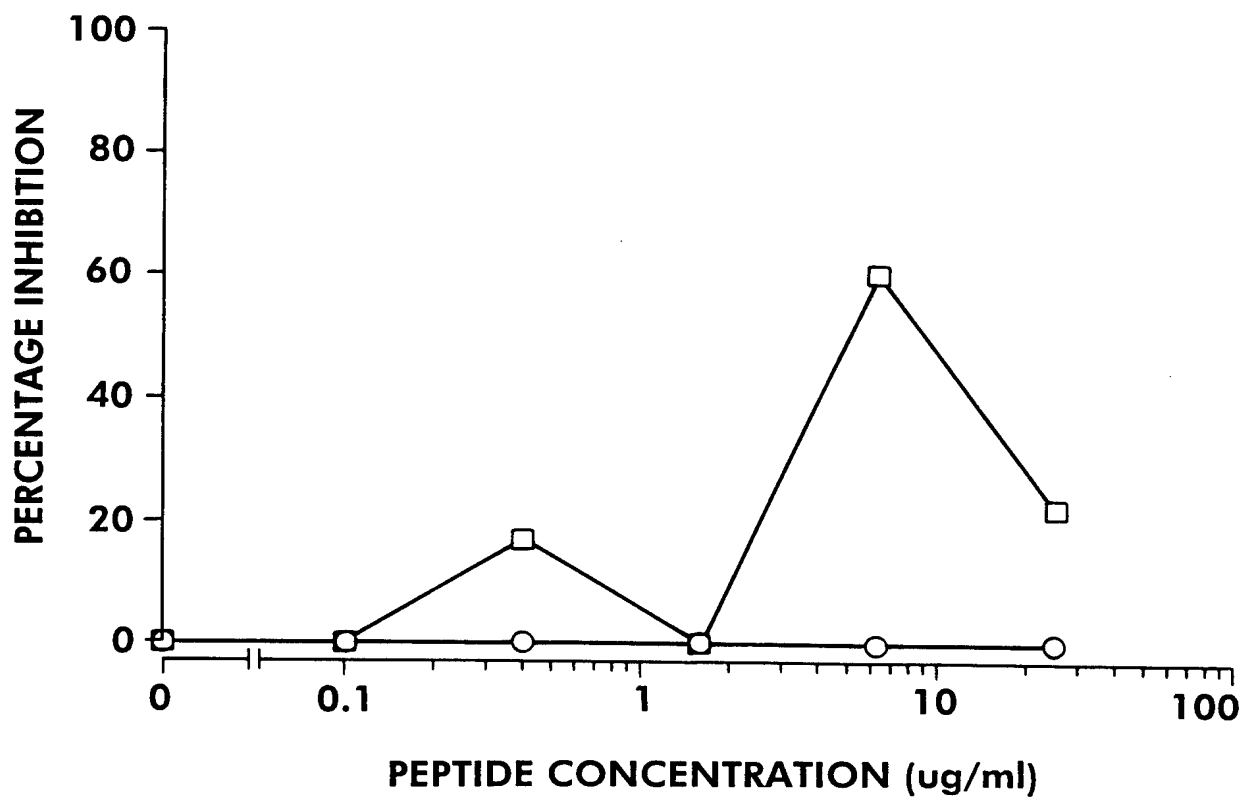
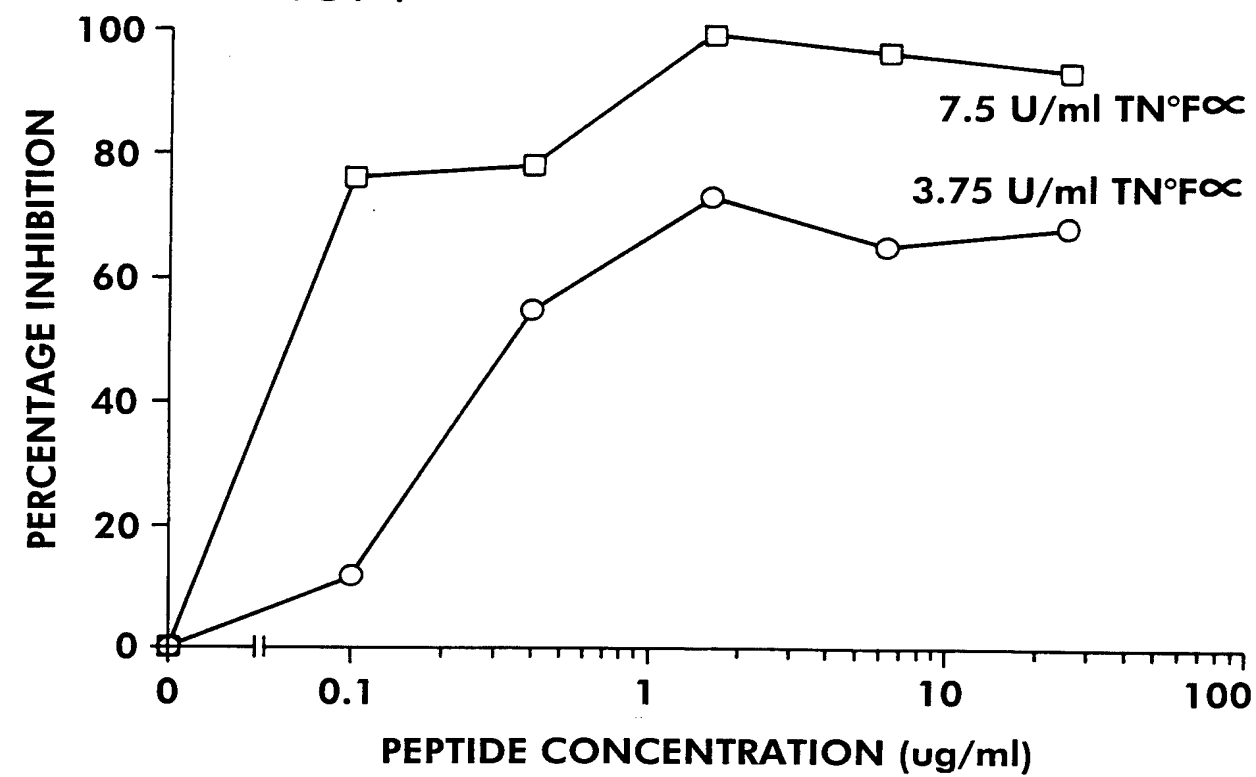


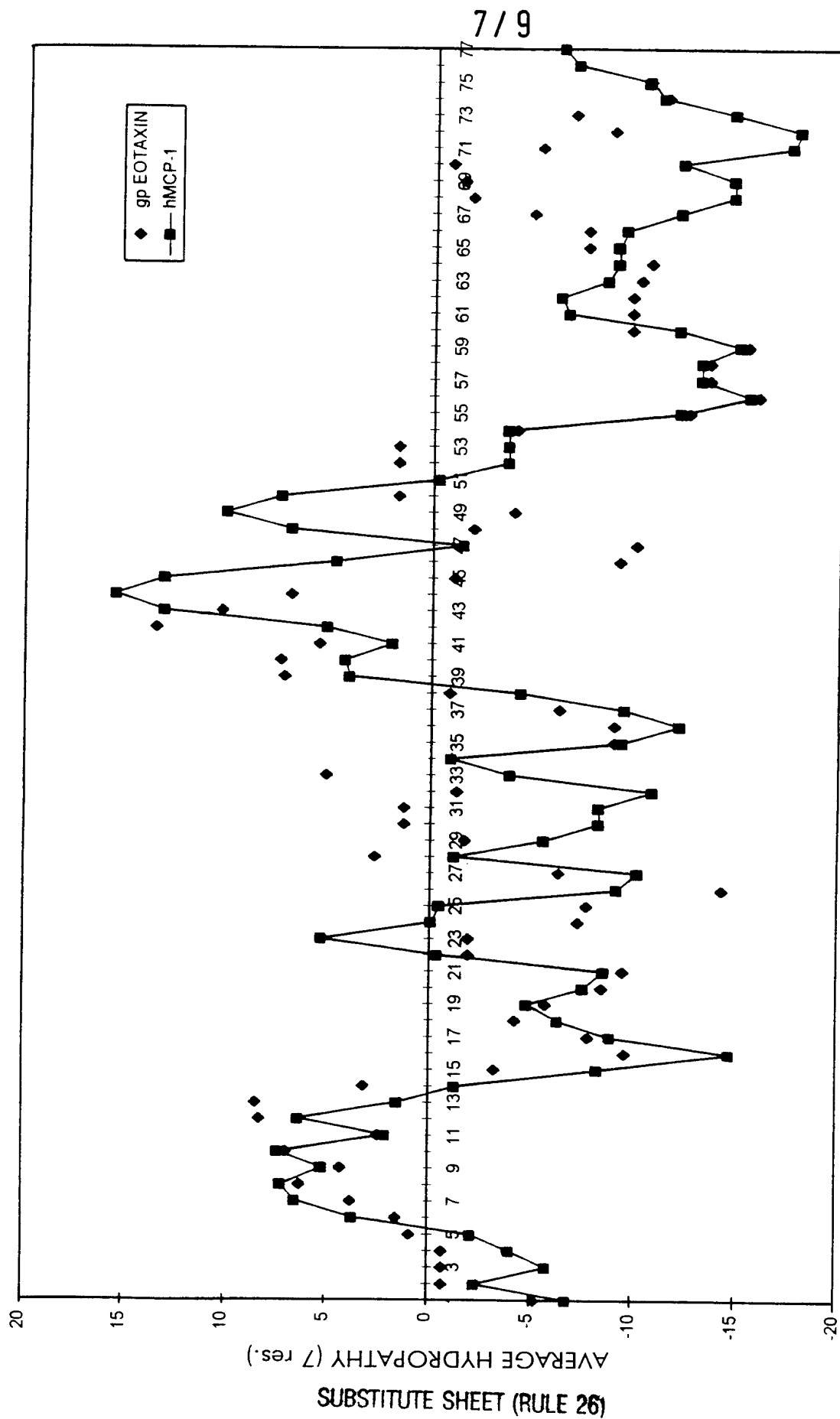
I A V S Y Q T K V TNFa 83-91  
 E Q N R I C T C R TNFR p55 91-99  
 D R D T V C G C R TNFRp75 91-99

FIG. 6

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FIG. 7





RESIDUE No.

FIG. 8



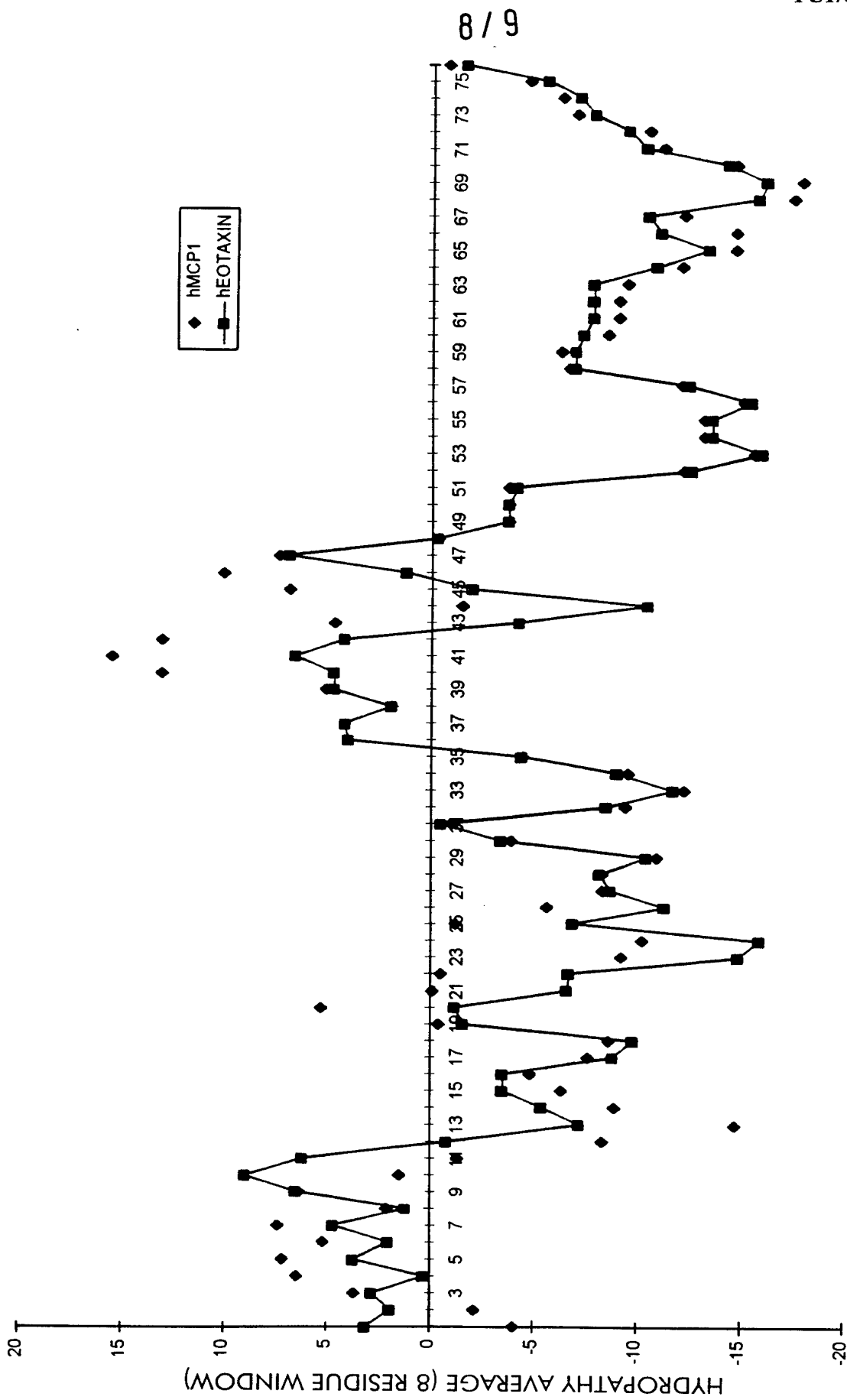
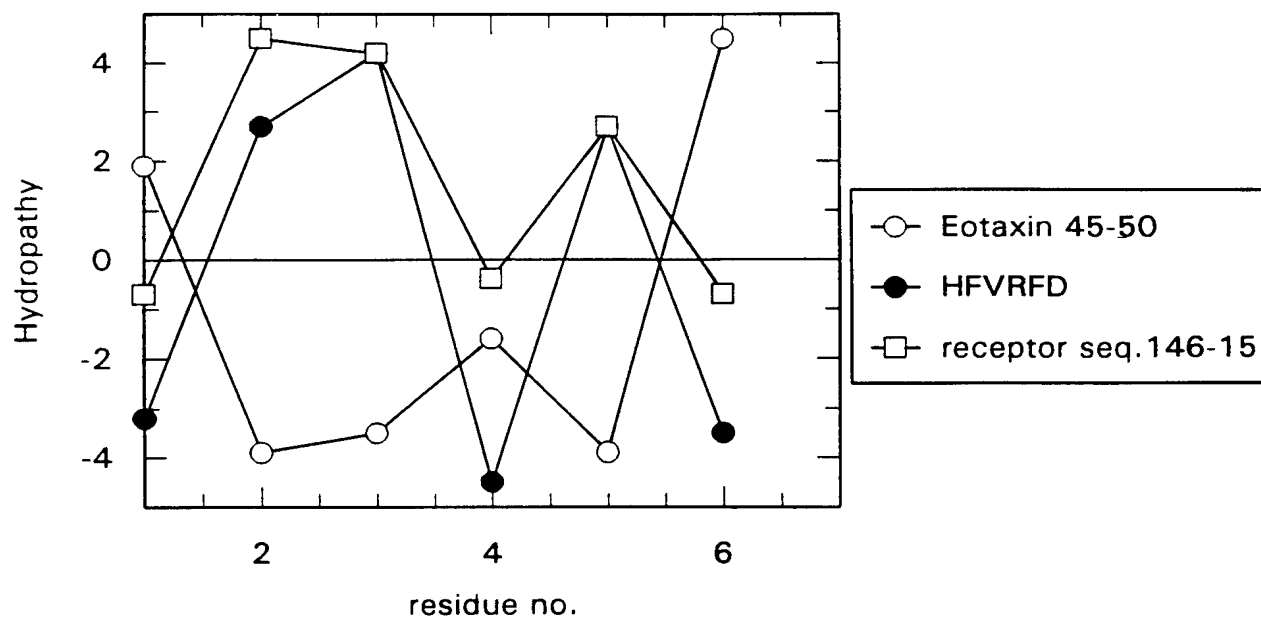


FIG. 9

RESIDUE NUMBER

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I K P D K M gp EOTAXIN 45 - 50  
 H F V R F D antisense peptide  
 T I V G F T CCCKR3 146 -152

FIG. 10

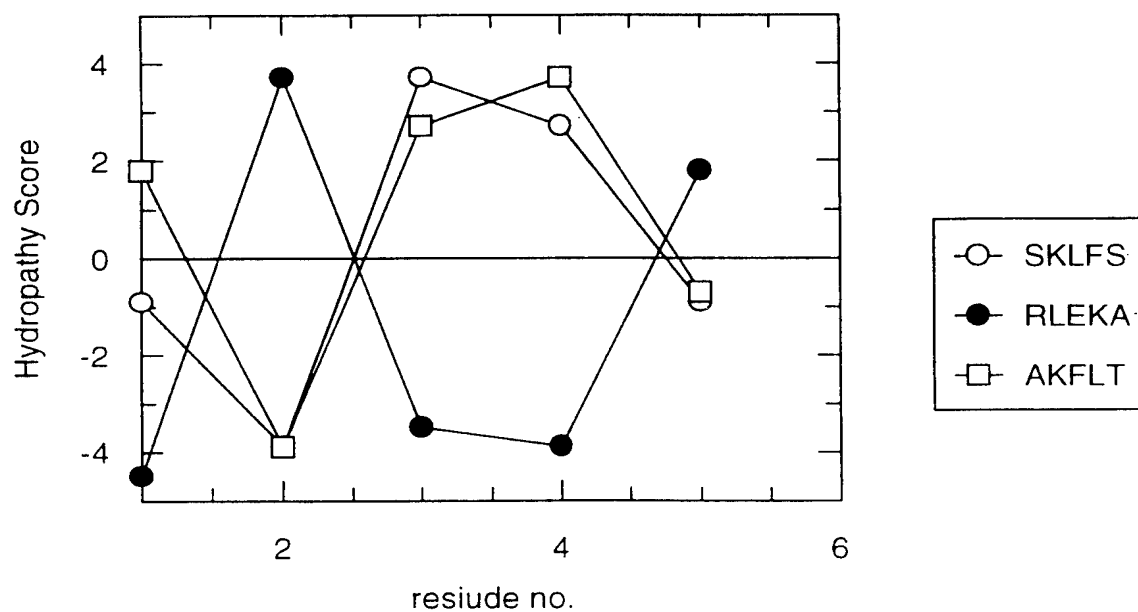


FIG. 11